Cell division: **The renaissance of the centriole**

Wallace F. Marshall and Joel L. Rosenbaum

Centrioles are located at the center of the cytoskeleton and duplicate exactly once per cell cycle. Recent studies suggest that centrioles are required for the organization of a functional centrosome and that centriole assembly requires both γ- and δ-tubulin.

Address: Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520, USA.
E-mail: Wallace.Marshall@yale.edu

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The centriole, a cylindrical array of nine triplets of microtubules found at the core of the microtubule-organizing organelle known as the centrosome, is one of the most beautiful, fascinating, and mysterious cellular structures. Despite great interest in centrioles due to their remarkable ninefold symmetry, their central location within the cytoskeleton, and their precise doubling during cell division, the function and duplication of these structures remain poorly understood.

Centriole function

One function of centrioles is not mysterious: these structures become basal bodies, directing the assembly of cilia and flagella. One normally associates cilia and flagella with protists like *Paramecium*, but human tissues contain identical structures, such as the flagella of sperm cells or the cilia on the epithelia of the respiratory and reproductive systems. Cilia also play sensory roles: in the eye, for example, the outer segments of rods are modified cilia. Recently, cilia were shown to be essential for the development of left–right asymmetry in mice [1]. The ubiquity of cilia means that basal bodies, which determine the number and position of cilia, have relevance extending far beyond the ciliated protozoa.

But our interest in centrioles does not end there. The centrosome, consisting of a pair of centrioles embedded in a mass of amorphous, pericentriolar material, is the primary microtubule-organizing center of animal cells, and forms the poles of the spindle during mitosis. The location of centrioles at the core of the centrosome hints at a role for these structures in cell division. Specifically, centrioles might act as organizing centers to recruit microtubule-nucleating factors, such as γ-tubulin, into a single focus in the cell, thus creating a centrosome. To simplify discussion, we use the general term microtubule-nucleating material to describe the factors involved in microtubule nucleation, which include γ-tubulin, other components of the γ-tubulin ring complex, and possibly pericentrin.

The hypothesis, then, is that centrioles provide a discrete scaffold around which all of the microtubule-nucleating material in the cell is collected into a single focus to form the centrosome. In some organisms, such as higher plants, this material is not organized in a discrete focus but instead is dispersed over a vast region of the cell. Such organisms do not have centrioles, supporting the view that the primary function of centrioles in cell division is to recruit this material into a focus.

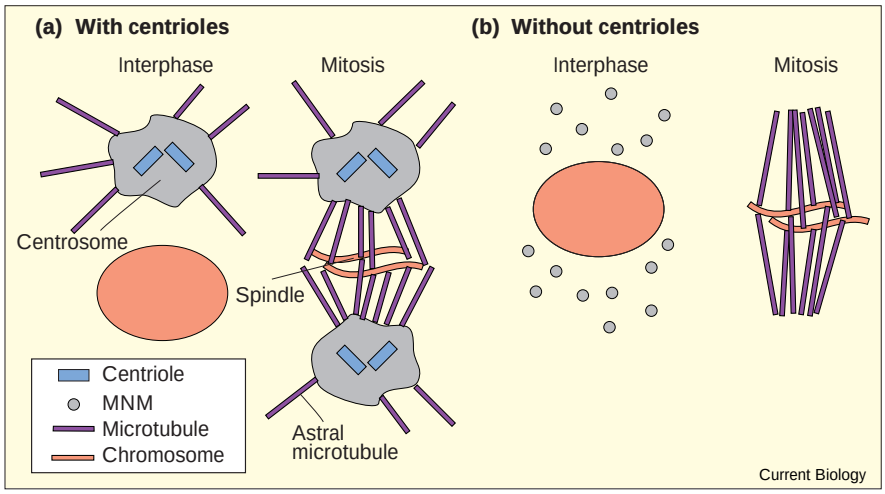
Direct evidence that centrioles organize the centrosome was recently obtained by Bobinnec *et al.* [2], who loaded HeLa cells with antibodies against polyglutamylated tubulin. Antibodies to this tubulin isoform, which is found only in centrioles, caused centrioles to disassemble. Remarkably, when centrioles were disrupted in this way, the microtubule-nucleating material became dispersed throughout the cytoplasm, and no functional microtubule-organizing centers were present. Eventually, the antibody degraded and centrioles re-formed and, when they did, the nucleating material re-associated around them to generate microtubule-organizing centers. Centrioles thus seem to be necessary for assembling this material into discrete foci.

The role of centrioles in cell division is sometimes questioned because bipolar spindles can form in cells that lack centrioles, implying that centrioles are not involved in centrosome assembly. This argument assumes that centrosomes are necessary for the formation of a spindle. But it is now known that, without centrosomes, chromosomes can themselves nucleate a microtubule array that self-organizes into a bipolar spindle [3]. So, one can argue that centrioles are in fact necessary to recruit microtubule-nucleating material to form a centrosome, but that, in the absence of centrioles, spindles can form via a default pathway that does not require a centrosome.

A recent study in the fly *Sciara coprophila* [4] confirms this idea. In normal *Sciara* development, the oocyte contains virtually all of the proteins and organelles needed for development, except for the centriole, which is provided by the sperm. Unfertilized *Sciara* oocytes can develop parthenogenetically and, although they have no foci of microtubule-nucleating material, multiple rounds of nuclear division still take place via bipolar spindles. It has now been shown that these spindles assemble by growing out from the chromosomes [4], a mechanism that would not require centrosomes [3]. These spindles, however, show defects in positioning and lack astral microtubules [4], which are required for spindle orientation and are normally nucleated by the centrosome. The absence of astral

Figure 1

Role of centrioles in animal cell division. (a) When centrioles are present they recruit the microtubule-nucleating material (MNM) into a discrete centrosome. In mitosis, the centrosomes nucleate the spindle but also nucleate astral microtubules which position the spindle within the cell. (b) In cells without centrioles, microtubule-nucleating material is dispersed. In mitosis, there are no centrosomes but a bipolar spindle can self-organize around the chromosomes. This acentriolar spindle lacks astral microtubules, however, and cannot be properly positioned, leading to aberrant cell division.



microtubules confirms that the centriole-less embryos lack centrosomes. Thus, the centriole-less mitosis of *Sciara* provides additional evidence that centrioles are needed to recruit microtubule-nucleating material to form a centrosome. A genetic study in algae reached a similar conclusion [5]: *bld2* mutant cells of the unicellular alga *Chlamydomonas reinhardtii* lack centrioles but still form bipolar spindles, possibly by the same centrosome-independent pathway as that used in *Sciara*. Although spindles do form in *bld2* mutants, their positioning relative to the cleavage furrow is randomized, causing aberrant cytokinesis and cell division. The *bld2* cells also have disorganized microtubule rootlets [5] — the algal equivalents of astral microtubules.

Taken together, these studies suggest that centrioles are required for assembling a functional centrosome that is capable of nucleating astral microtubules (Figure 1). Although bipolar spindles can still form in the absence of centrioles, they form by a centrosome-independent default pathway [3], lack astral microtubules [4], and cannot be properly positioned relative to the cleavage furrow [5]. Clearly, the idea that centrioles are important for cell division is a concept whose death has been greatly exaggerated.

Centriole duplication

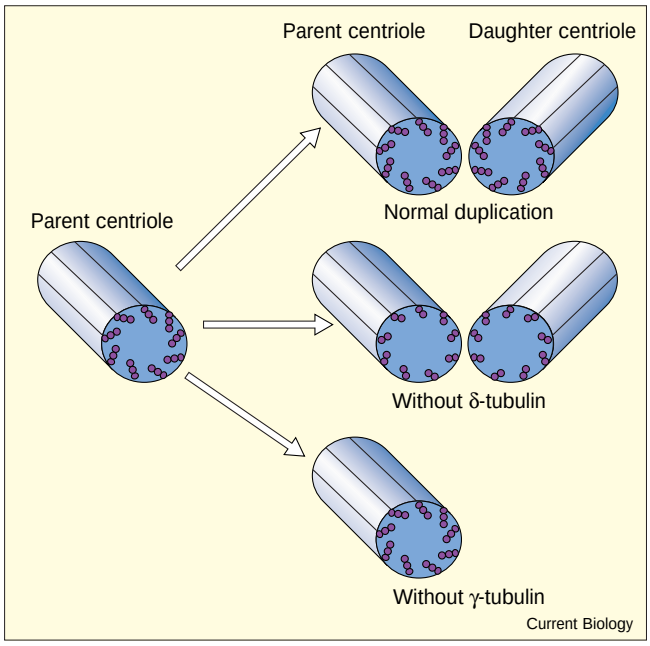
Even more enigmatic than centriole function is centriole duplication. Centrioles are the only organelles besides chromosomes that duplicate precisely once per cell division. Exactly one new, 'daughter' centriole forms adjacent to and at right angles with each pre-existing centriole. While this suggests that the pre-existing centriole acts as a template for the formation of the daughter centriole, the role of pre-existing centrioles in forming new centrioles remains unclear. In some cell types, such as oocytes laden with vast stockpiles of centriole components, centrioles can form *de novo* in the absence of pre-existing centrioles, but when centrioles are surgically removed from somatic cells these structures cannot regenerate [6]. Thus, the role of pre-existing centrioles in centriole assembly remains controversial. To some extent, we owe this controversy to a lack of molecular information about centriole duplication. Recently, two studies of basal bodies have begun to pry open the door of this black box.

One way to identify molecules involved in centriole duplication is the genetic approach, whereby mutants that are incapable of centriole duplication are identified and the gene(s) responsible for the phenotype isolated. Yeast has long been the favoured model organism for such genetic analysis, owing to its experimental tractability. Unfortunately, although the yeast spindle pole body is functionally analogous to the centrosome of animal cells, its structure and molecular composition are quite different. In particular, the yeast spindle pole body does not contain any microtubule-based structures that might correspond to the centriole. Thus, although yeast represents an exceedingly valuable genetic system for studying the cytoskeleton, it is not directly applicable to the study of centrioles *per se*.

Instead, some laboratories are turning to *Chlamydomonas*. Often called 'green yeast', *Chlamydomonas* is well suited for the genetic analysis of centriole duplication because it has similar genetic properties to yeast (it is haploid, divides rapidly, forms tetrads, and so on) but, unlike yeast, its basal bodies are identical to the centrioles of higher organisms. Moreover, as illustrated by the *bld2* mutation, basal body mutants of *Chlamydomonas* are viable, which facilitates their recovery and analysis.

To identify genes involved in centriole duplication, Dutcher and Trabuco [7] exploited the fact that *Chlamydomonas* swims using flagella, the formation of which

Figure 2



Dissecting centriole assembly. During cell division, each centriole gives rise to a daughter centriole consisting of nine triplet microtubules. In cells lacking δ-tubulin, centrioles still duplicate but each contains microtubule doublets instead of microtubule triplets. In cells lacking γ-tubulin, daughter centrioles do not form at all.

requires basal bodies. Screening for mutants with abnormal motility led to the identification of one mutant — termed *uni3* — which resulted in a phenotype where half of the cells lacked flagella [7]. Electron microscopy revealed that the basal bodies of the *uni3* mutant contained microtubule doublets instead of microtubule triplets [7]. The *UNI3* gene was cloned and found to encode δ-tubulin, a new member of the tubulin superfamily. Thus, *Chlamydomonas* genetics revealed a new tubulin required for a specific step of centriole assembly — addition of the third microtubule to each developing triplet in the centriole barrel (Figure 2). This gene evidently acts late in the centriole assembly pathway, because in *uni3* mutants defective centrioles continue to duplicate and segregate. Can genes involved in earlier steps be identified? Mutations in such genes would result in organisms that completely lack centrioles, and the *Chlamydomonas bld2* mutation, discussed above, may define such a gene.

Another way to identify centriole duplication genes is by reverse genetics, where the involvement of a particular gene is predicted, its expression is disrupted and the resulting effects are observed. One candidate duplication gene is that encoding γ-tubulin. This tubulin isoform localizes to the amorphous material surrounding the centrioles, where it nucleates microtubule formation, and is also found inside the lumen of the centriole at the proximal end [8], near the minus ends of the triplet microtubules. One model proposes that γ-tubulin is arranged on the outer wall of the parent centrioles where it acts as a template for daughter centriole formation [8].

Ruiz *et al.* [9] tested this model by injecting promoter-less DNA from the coding region of the γ-tubulin gene directly into the macronucleus of *Paramecium*. Injection of coding-region DNA is a recently described method for the trans-inactivation of endogenous copies of the corresponding gene in *Paramecium* [10]. Inactivation of γ-tubulin in this way caused a duplication defect, in that injected cells had only half the normal number of basal bodies [9]. In *Paramecium*, the cortex normally contains rows of paired basal bodies, each representing a parent and daughter basal body that remain associated. Cells containing inactivated γ-tubulin contained only rows of individual basal bodies instead of doublets, further indicating a duplication defect. Thus, γ-tubulin is clearly essential for centriole duplication (Figure 2).

These recent studies are a concrete step towards dissecting centriole duplication at the molecular level. As more such genes are identified and the genetic pathway of centriole duplication begins to emerge, the challenge will be to use this information and these mutants to ask what mechanistic role centrioles play in their own duplication and in the assembly of centrosomes during cell division. The recent progress outlined here suggests that we may soon reach a new level of understanding where we will still find centrioles beautiful and fascinating, but perhaps slightly less mysterious.

References

1. Nonaka S, Tanaka Y, Okada Y, Takeda S, Harada A, Kanai Y, Kido M, Hirokawa N: Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell* 1998, 95:829-837.
2. Bobinnec Y, Khodjakov A, Mir LM, Rieder CL, Eddé B, Bornens M: Centriole disassembly ***in vivo*** and its effect on centrosome structure and function in vertebrate cells. *J Cell Biol* 1998, 143:1575-1589.
3. Waters JC, Salmon ED: Pathways of spindle assembly. *Curr Opin Cell Biol* 1997, 9:37-43.
4. de Saint Phalle B, Sullivan W: Spindle assembly and mitosis without centrosomes in parthenogenetic ***Sciara*** embryos. *J Cell Biol* 1998, 141:1383-1391.
5. Ehler LL, Holmes JA, Dutcher SK: Loss of spatial control of the mitotic spindle apparatus in a ***Chlamydomonas reinhardtii*** mutant strain lacking basal bodies. *Genetics* 1995, 141:945-960.
6. Maniotis A, Schliwa M: Microsurgical removal of centrosomes blocks cell reproduction and centriole generation in BSC-1 cells. *Cell* 1991, 67:495-504.
7. Dutcher SK, Trabuco EC: The UNI3 gene is required for assembly of basal bodies of ***Chlamydomonas*** and encodes δ-tubulin, a new member of the tubulin superfamily. *Mol Biol Cell* 1998, 9:1293-1308.
8. Fuller SD, Gowen BE, Reinsch S, Sawyer A, Buendia B, Wepf R, Karsenti E: The core of the mammalian centriole contains γ-tubulin. *Curr Biol* 1995, 5:1384-1393.
9. Ruiz F, Beisson J, Rossier J, Dupuis-Williams P: Gamma-tubulin is necessary for basal body duplication in ***Paramecium***. *Curr Biol* 1999, 9:43-46.
10. Ruiz F, Vayssie L, Klotz C, Sperling L, Madeddu L: Homology-dependent gene silencing in ***Paramecium***. *Mol Biol Cell* 1998, 9:931-943.